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Comparison of Methods to Determine Rivaroxaban anti-factor Xa activity

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ABSTRACT

Background and Objectives: Rivaroxaban, a new oral anti-Xa agent, has been approved for use without routine monitoring, but the lack of a predictable drug level measurement may hinder the management of anticoagulated patients. The aims of the project were to correlate a Anti-Factor Xa assay using commercial calibrators and controls (Riva Activity) with serum drug levels analyzed by HPLC-MS/MS (Riva MS) in patients currently receiving rivaroxaban, and secondly, to correlate the PT/PTT, thrombin generation (CAT assay) and Thromboelastograph (TEG) with the Riva activity and Riva MS.

Methods: Recruited patients receiving rivaroxaban prospectively had a total of 3 blood samples taken at least 2 hours apart. Plasma was divided for measurement of PT/PTT, Riva activity, rivaroxaban HPCL-MS/MS, and thrombin generation. TEG activity was measured at one random time point for each patient. Correlation and linear regression evaluations were used to compare the different assays.

Results: The cases were 22 patients on rivaroxaban, age 56 + 12.6, and 10 healthy controls. There was a strong correlation between Riva activity compared to serum Riva MS ($r = 0.99$). We found a statistically significant correlation between PT/INR compared to serum measurements of Riva MS ($r = 0.68$) and anti-Xa activity ($r = 0.69$). The peak ($r = -0.50$) and lag time ($r = 0.57$) CAT correlated with Riva MS measurements. There was no correlation between Riva MS and PTT, TEG R, TEG MA, Endogenous Thrombin potential.

Conclusion: Riva anti-factor Xa activity assay measured with commercial calibrators and controls provides a reliable assessment of rivaroxaban serum levels for patients requiring measurement of anticoagulant activity. Correlation with other coagulation tests is not sufficiently strong to be used clinically.

Introduction

Vitamin K antagonists are no longer the only option for treating venous thromboembolism (VTE) or for preventing cardioembolic stroke [1–3]. This means that warfarin may no longer be the only choice for over 2 million patients with atrial fibrillation and the 600,000 new patients with VTE diagnosed in the United States every year [4,5]. Rivaroxaban, an oral direct factor Xa inhibitor, is a safe and effective alternative to warfarin currently indicated for the prevention of VTE post hip or knee replacement, for the prevention of cardioembolic stroke in patients with atrial fibrillation, and for the treatment of VTE.

While rivaroxaban has been proven safe and efficacious, there are certain situations where plasma concentrations of rivaroxaban must be known for clinical management of patients. This raises concerns that there is no readily available method to measure drug level or degree of anti-factor Xa activity. Such a method will be instrumental in monitoring compliance, potential medication failure, safety during peri- procedural anticoagulation management, and for the care of chronically anticoagulated patients. Activated partial thromboplastin time, thrombin time, and prothrombin time have been evaluated as surrogate measurements for rivaroxaban and found to have poor correlation [6]. Thromboelastogram [TEG] is often used in the operating room as a point-of-care device to evaluate the visco-elastic properties of whole blood. In contrast to clotting tests, TEG detects all phases of the coagulation process and fibrinolysis by measuring the change of elasticity during clotting and fibrinolysis and may therefore be more valuable to detect rivaroxaban induced variation. TEG measures clot formation by the tensile strength of the fibrin-polymer-platelet complex [7]. Similarly, there is a paucity of data correlating in-vivo rivaroxaban levels and TEG results Alternatively, high-performance liquid chromatography–tandem mass spectrometry [HPLC–MS/MS] can be used to measure rivaroxaban levels between 0.50 and 500 μg/L with excellent inter-assay accuracy, but the clinical applicability, slow turn-around-time and availability is limited [8]. To overcome these testing limitations, anti-factor Xa activity assay technology exists that,

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once validated, could be used by the clinical laboratory to perform rivaroxaban anti-factor Xa activity measurement.

The chromogenic anti-factor Xa assay is most commonly employed clinically to assess the anti-Xa activity of heparin-antithrombin, or low-molecular-weight heparin-antithrombin [9]. Rivaroxaban cannot be measured with the standard heparin, low-molecular weight heparin (LMWH) or hybrid calibrators and controls for measuring heparins with the anti-Xa assay. The activity of rivaroxaban is significantly higher than unfractionated heparin and LMWHs, obligating the necessity for specific calibrators and controls.

The aims of the project are to correlate a chromogenic anti-factor Xa assay using the Rivaroxaban Calibrators with the serum drug levels via HPLC-MS/MS (Riva MS) in patients currently receiving rivaroxaban, and secondly, to correlate the PT/PTT, thrombin generation (Calibrated Automated Thromograph or CAT assay) and Thromboelastograph (TEG) with the anti-factor Xa activity (Riva activity) and Riva MS. This study is designed to assess the clinically available methods to analyze rivaroxaban levels in patients.

Methods

This is a prospective cohort study. Twenty-two adults receiving rivaroxaban and 10 healthy controls were enrolled in the study. Inclusion criteria for rivaroxaban patients were: older than 18 years of age, taking rivaroxaban (no particular indication was differentially pursued), and able to provide informed consent. Exclusion criteria for rivaroxaban patients were: pregnant, or younger than 18 years of age. Controls were not receiving anticoagulation medication. Data collection included age, sex of patient, dose and time of oral intake of rivaroxaban, indication and length of rivaroxaban treatment, comorbidities and other current medications, in particular NSAID and aspirin use.

Recruited patients receiving rivaroxaban had a total of 3 blood draws. Two 3.2% sodium citrate collection tubes (blue top tubes 4.5 ml each) were collected using sterile technique at the time intervals specified below. Subjects were asked to remain available for 4 hours to complete sample collection. Plasma was obtained by standard clinical method of centrifugation [10]. Plasma was divided into 4 aliquots and stored in a non-defrosting freezer -20 C for batch measurement at end of study. In addition, a third citrate tube of whole blood was collected for TEG measurement as described below. Rivaroxaban levels were measured at three times points: at least 2 hours post ingestion of medication, and then 2 and 4 hours after initial measurement for a total of 66 samples from 22 patients We measured TEG activity at one random time point for each patient and the time post ingestion was recorded. For the 10 controls, 1 blue top tube (4.5 ml) was taken from each of the 10 healthy subjects not receiving anticoagulants. TEG was not measured on these samples.

Rivaroxaban levels were determined using an anti-factor Xa activity assay (STA®-Liquid Anti-Xa) using STA®-Rivaroxaban Calibrator and STA®-Rivaroxaban Controls on the STA-R Evolution® analyzer (Diagnostica Stago, Asnieres, France) at the completion of sample collection of all patients.

The PT/INR was determined using Neoplastine (ISI = 1.27) (Diagnostica Stago). The PTT assay was performed with STA® PTT-Automate (Diagnostica Stago, Asnieres, France).

Clot formation parameters were measured by thromboelastography (TEG) using the TEG Hemostasis System (Haemonetics Corporation, Braintree, MA, USA). Whole blood samples were obtained at one of the three sampling times. Within 2 hours of drawing the citrated whole blood specimen was activated with kaolin, and calcium citrate was added to obtain the thrombin-induced clot. Time to formation of the fibrin strand polymers (R time), and strength of the clot (MA) were measured. Comparisons were made to manufacturer's control values since standardized kits were used that established reference ranges, and level 1 and 2 controls were run before each specimen.

Thrombin generation was performed on the CAT (Diagnostica Stago, Asnieres, France) using PPP-High reagent (5 pm tissue factor). STA® Rivaroxaban Calibrators (Diagnostica Stago, Parsippany, NJ), STA® Rivaroxaban Controls (Diagnostica Stago, Asnieres, France) and Normal Pooled Plasma (Precision Biologic, Dartmouth, Nova Scotia) were run on each plate with rivaroxaban patient and control patient plasmas. Each thrombin generation plate analyzed four rivaroxaban calibrators, two rivaroxaban controls and pooled normal plasma. Thrombin generation data was analyzed for peak height, endogenous thrombin potential (ETP- as a measure of area under the curve), velocity index, time to peak, lag time and start tail.

Riva MS, using manufacturer provided rivaroxaban standard, was performed on plasma following dilution with a basic solution containing stable labeled internal standards, incubation for 2 h, extraction via turbulent flow liquid chromatography and subsequent analytical chromatographic separation. An ABSciex API5000 triple quadruple mass spectrometer (ABSciex, Toronto, Canada), operating in positive electrospray ionization mode was used for detection. Quantification of analyte and internal standards was performed in selected reaction monitoring mode (SRM). The back-calculated amount of the rivaroxaban in each sample was determined from calibration curves generated by spiking known amounts of purified rivaroxaban into drug-free defibrinated plasma from 5.0 – 1000 ng/mL.

Statistical analysis was performed using SAS (Version 9.2). A level of $p < 0.05$ was considered statistically significant. Categorical variables were expressed as percentages and continuous variables as mean ± standard deviation. The relationship between Riva-MS, rivaroxaban anti-factor Xa activity and the other coagulation variables were determined by Pearson's correlation. Additionally, the agreement of the Riva MS versus Riva activity quantification is presented in a Bland Altman differences plot.

Results

There were 22 cases and 10 controls, (1 male, mean age 44). Table 1 summarizes the demographic data of the cases. Of the cases, two patients reported using concomitant NSAIDS: two taking aspirin and another three taking celecoxib. Three patients could not have TEG measurement completed due to technical limitations.

Table 2 summarizes the strength of the associations between measures of coagulation tested and HPLC-MS/MS. There was a strong correlation between Riva activity compared to plasma Riva MS ($r = 0.99$). This was found at a wide range of concentrations of rivaroxaban from 25 ng/ml to 460 ng/ml . (Figs. 1 and 2) The mean difference between the two tests was only 4.3 ng/mL (95% Confidence Interval -25.3 to 33.8). The variability within the 95% confidence interval is unlikely to be clinically relevant. The lower limit of the assay is established by the lowest value of the calibrators, which is 25 ng/ml. In addition, only 4 patients had Riva activity greater than 300 ng/ml. Three of these patients took their medication the same morning of the sample collection. The other patient took her medication late evening the

Table 1
Demographic data.

	Cases N = 22
Male:Female	5:17
Age (mean + SD)	56 + 12.6
Race Caucasian	19
Rivaroxaban dose 10 mg	3
15 mg	3
20 mg	16
Indication	8
Atrial fibrillation	13
Venous thromboembolism	1
Peripheral arterial disease	